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BRIEF COMMUNICATION

ISOLATION OF *Salmonella enterica* AND SEROLOGIC REACTIVITY TO *Leptospira interrogans* IN OPOSSUMS (*Didelphis virginiana*) FROM YUCATÁN, MÉXICO

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SUMMARY

The presence of *Salmonella enterica* and serologic evidence of infection by *Leptospira interrogans*, were detected in the opossum *Didelphis virginiana* in a semi-urban locality of the Yucatán State, México. Ninety-one opossums were captured during the period April 1996 and May 1998. From a total of 17 feces samples, four *Salmonella enterica* subsp. *enterica* serotypes (Sandiego, Newport, Anatum, and Minnesota), and one *Salmonella enterica* subsp. *arizonae* serovar O44:_{7A,Z23:-} were isolated. Some opossums presented mixed infections. From 81 sera samples, four (4.9%) were positive to antibodies to *Leptospira* serovars *pomona* and *wolfii*. Both animals infected with *Salmonella enterica* and those serologically positive to *Leptospira interrogans* were captured in peridomestic habitat. Opossums infected with *Salmonella enterica*, were captured in dry season, and those seropositive to *Leptospira interrogans* during the rainy season. The implications of infection and reactivity of these zoonotic pathogens in *D. virginiana* in the Yucatan state are briefly discussed.

KEYWORDS: *Didelphis virginiana*; *Salmonella enterica*; *Leptospira interrogans*; Yucatán; México.

Opossums of the genus *Didelphis* have been frequently found infected with several zoonotic diseases⁷. *Didelphis virginiana* occurs widely in México including the Yucatán Peninsula where this opossum is abundant in the peridomestic habitat, thus bringing a risk to transmit zoonotic diseases such as Chagas disease to the human populations⁸. In this paper, we report for the first time in México data on the infection by *Salmonella enterica* and the seroprevalence of antibodies against *Leptospira interrogans* in *D. virginiana*. This information constitutes a contribution to the knowledge of the epidemiology of zoonotic diseases in México, where in spite of the abundance of this mammal in peridomestic habitat, its role in the epidemiology of many zoonotic diseases has not been evaluated.

The data of infection by *S. enterica* and *L. interrogans* in opossum, were obtained during a populational study of this mammal to evaluate its importance as a reservoir of *Trypanosoma cruzi*, the etiologic agent of Chagas disease⁸. Opossums were captured from April 1996 to May 1998 in Dzidzilché (21°08'51.76" and 89°41'28.24"), a semiurban small village located 25 km northwestern Mérida, the capital of the Yucatán State, México (Fig. 1). Thorny and low forest, and scarce remains of low deciduous forest characterized the vegetation of the study area. Many abandoned sisal fields dotted the surroundings of the village. Fruit trees such as *Mangifera indica* (mango), *Manilkara achras* (gumtree), *Spondias purpurea* (plum), *Citrus aurantium* (orange), and *Carica papaya*

(papaya), were common in the courtyards. The climate was predominantly warm and humid, with a mean annual temperature of 25.4 °C. During the study period, the rainy season ranged from June to October, and the average annual rainfall was 449 mm.

The capture of opossums was carried out following the capture-marking-recapture scheme in a grid of 4 x 4 Tomahawk livetraps set at 250 m interval. The grid included all human households of the village and comprised an area of 156 ha. For comparative purpose, traps set less than 50 m from households were considered in peridomestic habitat. Nine capture periods of three weeks each, were carried out. During the first week, animals were captured, marked using a 2-mm ear punch, and released in the site of capture. The capture was interrupted in the following week to allow dispersion of marked animals. During the third week, the steps described in the first week were repeated. After a 51 days delay, a new capture period was then initiated.

The opossums were sexed and classified in two age groups (juvenile and adult) on basis of reproductive condition, eruption and wearing of teeth⁶. Opossums were anesthetized via intramuscular injection with ketamine (20 mg/kg) (Ketaset®, Ford Dodge, Iowa, USA). When available, fresh feces were recollected during the handling of the animal, put on a plastic bag and stored at 4 °C. 3-5 ml of blood were extracted by cardiac puncture, from which serum samples were obtained.

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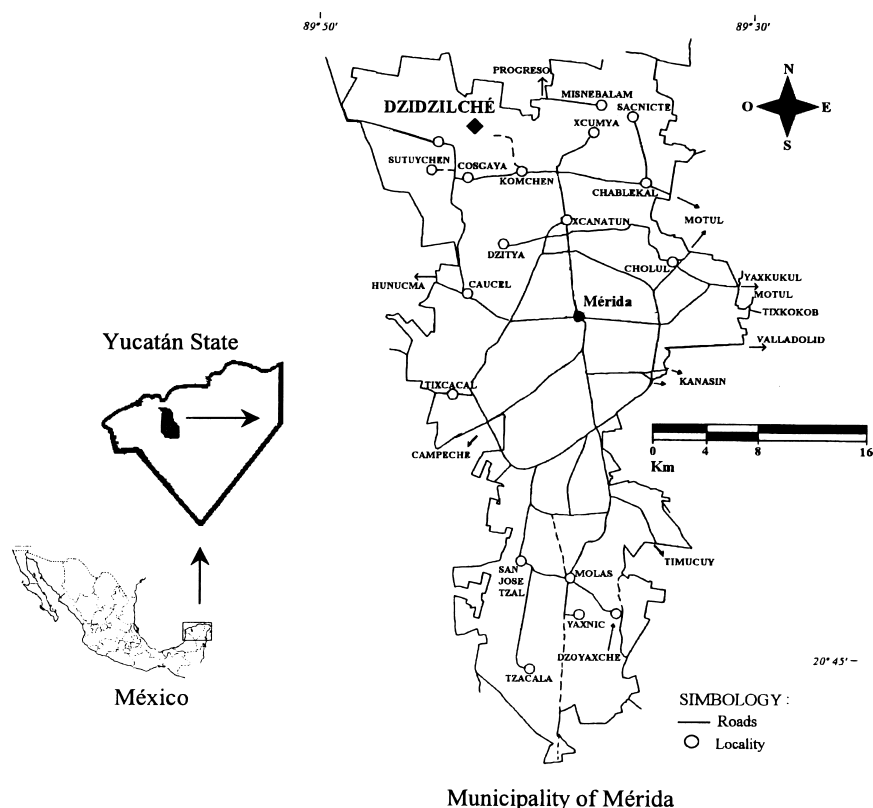


Fig.1 - Map showing the study area (•) in Northwest of Yucatán State, México.

Opossum's feces samples were plated in MacConkey agar (DIFCO Laboratories, Detroit, MI, USA) and inoculated in Tetrathionate broth (DIFCO). The agar was incubated at 37 °C for 24 hrs; Tetrathionate broth was incubated at 37 °C for 12-18 hrs; after that, a loop was transferred to a *Salmonella*-*Shigella* agar (DIFCO) plate, which was incubated at 37 °C for 24 hrs. Colonies suspected of being *Salmonella*, were examined by biochemical test, according to the methodology described by BALLOWS *et al.*, 1991¹. For *Salmonella* serotyping both rapid slide test (agglutination) for somatic antigen determination, and macroscopic tube test (agglutination) for flagellar antigens titration², were performed at the Instituto de Diagnóstico y Referencia Epidemiológicos (INDRE) of Mexican government. Serovar representation was done according to the criteria of MINOR & POPOFF³.

Eighty-one serum samples were tested by the Microscopy Agglutination Test (MAT)⁵, using antigens of *Leptospira interrogans* of the serovars *canicola*, *icterohaemorrhagiae*, *grippotyphosa*, *pyrogenes*, *pomona*, *wolffi*, *tarassovi*, *bratislava*, *panama*, and *hardjo*. These antigens were kindly donated by the Veterinary Medicine Faculty of the Hokkaido University, Japan. Antibody titers of 1:100 or higher were considered positive.

Ninety-one opossums (53 females and 38 males) were captured during the nine capture periods, with higher capture in dry season months (Table 1). Twenty-four opossums (18 females with embryos) were recaptured in peridomestic traps, 83% of which was during dry season months (data not shown).

Five opossums out of 17 (29.4%) studied were found infected with four *Salmonella enterica* subsp. *enterica* serotypes (Sandiego, Newport, Anatum, and Minnesota), and one *Salmonella enterica* subsp. *arizonae* serovar O44:ZA,Z23:- which was differentiated from *Salmonella enterica* subsp. *houteanae* by four biochemical reactions; ONPG test, Malonate utilization, growth in KCN medium, and Mucate fermentation¹. Two opossums had mixed infections. One was previously negative when it was captured in February 97 (Table 1). All infected opossums were females with embryos in their pouch, captured in peridomestic habitat during capture periods corresponding to the driest and hottest months of the corresponding year (Table 1).

From 81 serum samples tested, antibodies against *L. interrogans* were found in four females (4.9%) (two adults and two juveniles), three of them had serologic reactivity against the serovar *pomona* and one against the serovar *wolffi* (Table 1); titers ranged from 1:100 to 1:400. One of the two adult females was serologically negative when it was first captured in April-May 96. The four opossums were captured in peridomestic traps during the rainy season of 1996 (Table 1).

Reports on infection of *Salmonella* and *Leptospira* in opossums have been previously documented in the American Continent⁷, but as far as we know, our results represent the first report of *Salmonella enterica*, and serologic reactivity against *L. interrogans* in opossums from México. One interesting finding in this study was that all the opossums infected with *Salmonella enterica* were captured during the dry season, which is in agreement with a previous study in Panamá showing that the highest

Table 1

Distribution and prevalence of *Salmonella enterica*-infected and *Leptospira interrogans*-serologically reactive opossums during the capture periods from April 1996-May 1998 in Dzidzilché, Yucatán, México

Capture Months/Year	Climatic season	Animals captured	<i>Salmonella</i> N. feces/Positive/(%)	<i>Leptospira interrogans</i> N. sera/Positive/(%)
4-5/96	D	14	5*/2 ^{S,N} /40	10/0/0
6-7/96	R	8	2/0/0	5/1 ^p /20
8-9/96	R	4	2/0/0	4/1*/25
11/96	R	7	-/-/-	12*/2 ^p /16.6
2/97	D	10	1/0/0	13/0/0
4-5/97	D	10	4*/2 ^{AM,AZ} /50	6/0/0
9/97	R	9	-/-/-	7/0/0
2-3/98	D	12	3/1 ^Z /33.3	11/0/0
4-5/98	D	17	-/-/-	13/0/0
Total		91	17/5/29.4	81/4/4.9

D = Dry, R = Rainy. *Salmonella enterica* subsp. *enterica* serotypes: S = Sandiego, N = Newport, A = Anatum, M = Minnesota. Z = *Salmonella enterica* subsp. *arizonae*, AM and AZ = mixed infections. *Leptospira interrogans* serovars: p = Pomona, w = Wolffi. * includes samples of recaptured opossums previously uninfected or serologically negative.

incidence of infection by this bacteria among mammals (including opossums) was also observed during the dry season³. On the other hand, seropositive opossums to *Leptospira* were all captured in the rainy season coinciding with previous epidemiological studies in Yucatán showing a higher prevalence of human cases of *Leptospirosis* during this period of the year⁹. In a previous study, we showed that the ecological characteristics of *D. virginiana* in Yucatán constituted key factors that influenced the seasonality of the peridomestic transmission of *T. cruzi* among opossums and the insect vector: *D. virginiana* colonized rural peridomestic habitat with higher adult densities and reproductive activity during the dry season, and their population presented a predominance of juveniles (susceptible animals) in the rainy season⁸ (July to November). In spite of the reduced number of sample analyzed in this preliminary report, our results point out the need for systematic research to evaluate the role of opossums as carriers of those two zoonotic pathogens in the state of Yucatán.

RESUMEN

Aislamiento de *Salmonella enterica* y reactividad serológica a *Leptospira interrogans* en tlacuaches (*Didelphis virginiana*) de Yucatán, México

La presencia de *Salmonella enterica* y evidencia serológica de infección por *Leptospira interrogans* fueron detectadas en tlacuaches de la especie *Didelphis virginiana* capturados en una localidad semi-urbana del estado de Yucatán, México. Se capturaron 91 marsupiales durante el período de abril de 1996 a mayo de 1998. De un total de 17 muestras de heces, se aislaron cuatro serotipos de *Salmonella enterica* subsp. *enterica* (Sandiego, Newport, Anatum y Minnesota) y una *Salmonella enterica* subsp. *arizonae* serovar O44:^{Z4,Z23:-}. En algunos tlacuaches se registraron infecciones mixtas. De 81 muestras de suero, cuatro (4,9%) presentaron reacciones positivas con los serovares *pomona* y *wolffi*, ambos pertenecientes al género *Leptospira*. Los tlacuaches con serología positiva fueron capturados en el hábitat peridomiciliar. Los

animales infectados con *Salmonella enterica* fueron capturados en los períodos de seca y aquellos que presentaron serología positiva para *Leptospira interrogans* en los meses lluviosos. Las implicaciones de estos hallazgos en el mantenimiento y transmisión de estos dos agentes zoonóticos en el estado de Yucatán, son brevemente discutidos.

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